

## Studies in venereal disease

### *II. Improved diagnosis of gonorrhoea by the parallel use of conventional and L-phase media for culture*

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The frequency of gonorrhoea has increased rapidly in recent years. From 1960 to 1970 the number of cases with gonorrhoea increased from about 18,000 to 40,000 in Sweden (Juhlin and Wallin, 1972). In the United States about 620,000 cases were reported in 1971, although the real incidence of gonorrhoea was estimated to be about 2 million cases (Rudolph, 1972). This has made gonorrhoea one of the most prevalent infectious diseases, second only to measles and the common cold.

Several reasons for this increase have been given. Asymptomatic gonorrhoea is now encountered more frequently than before, especially in women (Pariser, 1972). More penicillin-resistant strains have been isolated in some areas (Sparling, 1972; Holmes, Johnson, and Floyd, 1967). Induction of L-phase organisms by conventional treatment has been suggested by Holmes, Johnson, Floyd, and Kvale, (1967), and the isolation of the gonococcal L-phase from patients with acute venereal disease has been reported (Gnarpe, Wallin, and Forsgren, 1972).

The following study was made to evaluate the practical importance of pure L-phase organisms of *N. gonorrhoeae* in venereal disease. We also wanted to investigate whether the number of isolations could be increased by the use of media supporting growth of the gonococcal L-phase.

#### **Material and methods**

##### *Patients*

All male and female patients attending the Venereal Diseases Department of the Dermatological Clinic of the University Hospital in Uppsala for a suspected venereal disease were included. Between September and December, 1972, 769 patients were investigated with conventional and L-phase cultures.

##### *Clinical and microbiological procedure*

Specimens were taken from the male urethra and from the cervix with charcoal-coated cotton swabs and immersed in a protective medium as described in our earlier report (Gnarpe and others, 1972). On arrival at the laboratory, all specimens were first cultured on conventional media with and without the addition of VCN-inhibitor (BBL). All specimens were then inoculated on media especially adapted for isolation of the gonococcal L-phase. All plates were incubated at 37°C. in 5 per cent. CO<sub>2</sub>. Conventional cultures were read after 48 hrs; L-phase cultures were read daily under a stereomicroscope (Zeiss, stereo 4). When L-phase organisms were discovered they were cut out and subcultured until reversion occurred as described earlier. A final identification of *N. gonorrhoeae* was made with a fluorescent antibody technique by the method of Danielsson (1965) but with the FA-serum produced as suggested by Forsum (1973).

##### *Media*

The conventional medium used had the following composition: Gc agar base (BBL) 36 g., isovitalax 10 ml. (BBL), haemoglobin (BBL) 20 g., aq. dest. to 1,000. Plates with the above composition were used in parallel with plates containing VCN-inhibitor (BBL) 10 ml./l. added for suppression of contaminants. This medium has been used for several years in the laboratory with satisfactory results.

After extensive study, a new L-phase medium was developed from the previous one since this had proved unsatisfactory in some respects. The medium used in the present investigation had the following composition: brain heart infusion (Difco) 37 g., sucrose 30 g., and corn starch 1 g. were autoclaved in 390 ml. aq. dest. To this was added 50 ml. of 25 per cent. yeast extract (Jästbolaget, Stockholm), 150 ml. normal inactivated house serum, and 10 ml.

isovitalex (BBL), 10 g. haemoglobin (BBL) were boiled for 30 min. in 400 ml. aq. dest and the filtrate was added together with 8.09 g. Noble agar. This medium was found satisfactory for the culture of L-phase organisms of *N. gonorrhoeae*. L-phases of other bacteria will also grow on this medium, but it does not support the growth of mycoplasmas.

## Results

Conventional and L-phase cultures were made with specimens taken from a total of 448 men and 321 women at their first visit to the Venereal Disease Department. Conventional cultures gave a growth of gonococci from 86 men and from 76 women (Table). In parallel cultures on L-phase plates, gonococci were isolated from 66 of the 86 men (77 per cent.) and from 33 of the 76 women (43 per cent.). In those cases in which gonococci grew on conventional media only, the L-phase media usually showed a heavy overgrowth of urethral contaminants or of lactobacilli which made the L-phase plates difficult to read. Lactobacilli did not grow on the conventional media used.

TABLE *Isolation of N. gonorrhoeae from an unselected series of 448 men and 321 women with suspected acute venereal disease in cultures on conventional and on L-phase media*

Number of patients with positive cultures	Sex	
	Male	Female
On conventional and L-phase media	66	33
On conventional media only	20	43
On L-phase media only	9	2
Total	95	78

Gonococci from 75 men and 35 women were isolated on L-phase media (Table). The parallel conventional cultures were negative in nine men and two women. Gonococci grew in mixed bacterial and L-phase cultures in only two of these eleven cases, one man and one woman. In the remaining nine cases the gonococci were discovered in the bacterial phase of growth.

The gonococcal L-phase was isolated from nineteen of the 95 men and from thirteen of the 78

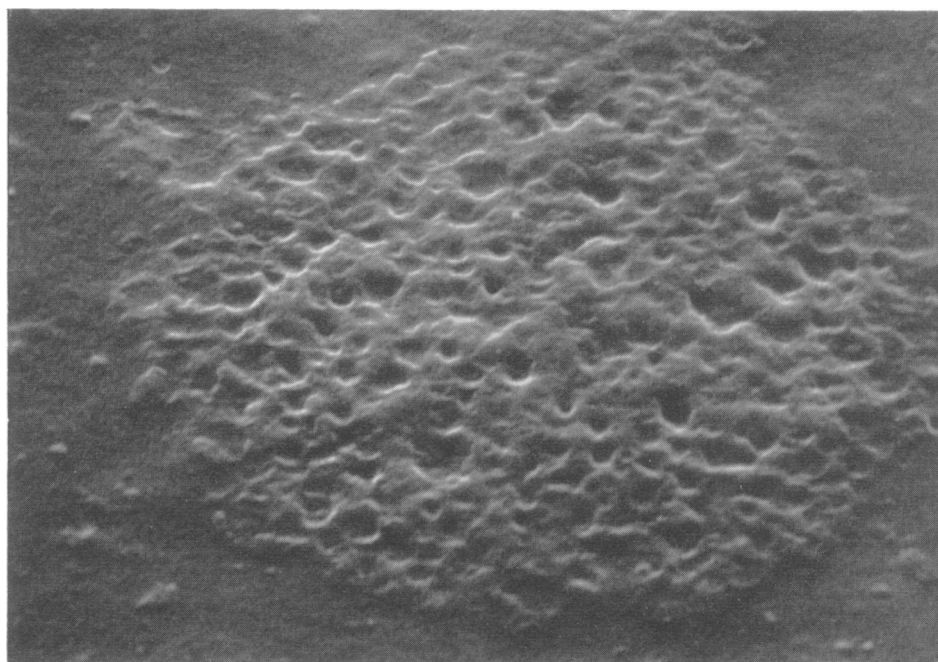


FIGURE *Scanning electron micrograph of a gonococcal L-phase colony 24 hrs after the first subculture on L-phase medium. The hollows in the colony represent collapsed vacuolized cells. Fixed in 2.6 per cent. glutaraldehyde + 5 per cent. sucrose; alcohol-dehydrated.  $\times 950$*

women with gonorrhoea. In all cases the L-phase colonies (Figure) were mixed with ordinary bacterial phase colonies. No stable L-phase organisms were isolated.

### Discussion

No certain microbial aetiology can be detected in many cases of acute urethritis. In our earlier report, we have shown that L-phase organisms of *N. gonorrhoeae* could be the aetiological agent, at least in isolated cases (Gnarpe, Wallin, and Forsgren, 1972).

Our present results show that L-phase organisms of *N. gonorrhoeae* may be isolated from as many as 20 per cent. (19 of 95) of all male patients with gonorrhoea. It is not likely that the L-phase organisms were induced *in vitro* as no inducing agents were used. All of the isolated gonococcal L-phase organisms grew in mixed bacterial and L-phase cultures. In some of these cases it is possible that a pure growth of L-phase organisms *in vivo* rapidly reverted to bacterial growth *in vitro* (9 of 95 men and 2 of 78 women) as no growth occurred on conventional media despite transportation to the laboratory in protective media.

We found a pronounced sex difference in the isolation rate on L-phase compared to conventional media. This was probably due to microbial interference caused by the heavy growth of lactobacilli in the cultures from women. This problem will be further investigated using a modified L-phase medium suppressing the growth of lactobacilli.

The results of this investigation indicate that about 10 per cent. (9 of 95 men) of all male cases of gonorrhoea may escape an accurate diagnosis if only conventional methods are used. It seems likely that these 'missed' cases may contribute to the spread of gonorrhoea and that the control of the disease would be aided if cultures were also made on L-phase media.

### Summary

Parallel cultures on conventional and L-phase media

for *N. gonorrhoeae* were made with specimens from 448 men and 321 women with suspected acute venereal disease. Conventional culture resulted in a growth of gonococci from 86 men and 76 women. The use of L-phase media gave an increase in isolation rate of 10 per cent. in the male group and 2 per cent. in the female group. L-phase organisms were isolated from 20 per cent. of all men and 17 per cent. of all women.

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### Etudes vénéréologiques.

II. Meilleures possibilités de diagnostic de la gonococcie grâce à l'utilisation en parallèle, pour la culture, de milieux conventionnels et de milieux pour phase L

### SOMMAIRE

Des cultures en parallèle sur des milieux conventionnels et des milieux pour phase L pour *N. gonorrhoeae* furent faites à partir de spécimens provenant de 448 hommes et de 321 femmes suspects de maladies vénériennes aiguës. Avec la culture conventionnelle, le gonocoque poussa chez 86 hommes et 76 femmes. L'emploi de milieu pour phase L permit un gain de positivité de 10 pour cent chez les hommes et de 2 pour cent chez les femmes. Des organismes en phase L furent isolés chez 20 pour cent de l'ensemble de hommes et chez 17 pour cent de l'ensemble des femmes.